Investigation of repeated vaccination as a possible cause of glomerular disease in mink

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Abstract

The search for antigens capable of causing immune-complex-mediated glomerulonephritis continues. Modified live-virus vaccines commercially available for veterinary use are a possible source. In this study, repeated vaccination of mink with live-virus vaccines was investigated as a model for vaccine-induced glomerular injury. Three groups of 10-wk-old mink, 15 per group, were vaccinated once with 4-way vaccine against distemper, *Pseudomonas aeruginosa* infection, botulism and mink viral enteritis. Subsequently, all mink in each group each were vaccinated either with the 4-way vaccine, a monovalent canine distemper vaccine, or saline. Glomerular function was assessed at 2-wk intervals by determining the urinary protein:creatinine (P:C) ratio. Kidney sections taken at necropsy, 20 wk after the 1st vaccination, were examined by light and immunofluorescent microscopy for deposition of immunoglobulin and complement. There was no statistically significant difference between the treated and control groups based on average urinary P:C ratio medians. Light microscopic changes were detected in glomeruli, but Fisher's exact test showed no significant differences between any of the treatment groups. Deposition of immunoglobulin but not complement was significantly more frequent ($P < 0.05$) in the glomeruli of animals that received multiple injections of the 4-way vaccine than in the glomeruli of those given only the monovalent canine distemper vaccine or saline. These findings suggest that repeated vaccination may increase the glomerular deposition of immunoglobulin. Further studies are required to determine if the increased deposition of immunoglobulin contributes to the development of glomerular damage and to identify the antigens driving production of the deposited immunoglobulin.

Résumé

Les vaccins vivants modifiés disponibles commercialement sont une source potentielle d'antigènes capables d'induire une glomérulonéphrite à complexes immuns. La vaccination répétée de visons à l'aide de vaccin viral vivant fut étudiée comme modèle possible de dommages glomérulaires induits par la vaccination. Trois groupes de 15 visons par groupe, âgés de 10 semaines, furent vaccinés une seule fois avec un vaccin tétravalent contre le distemper canin, Pseudomonas aeruginosa, *le botulisme et l'entérite virale du vison. Par après, les visons de chaque groupe furent vaccinés 8 fois avec, respectivement, le même vaccin tétravalent, un vaccin monovalent contre le distemper canin, ou de la saline. La fonction glomérulaire fut évaluée à des intervalles de 2 semaines en déterminant le ratio protéine:créatinine. Des sections de rein, prélevées lors de la nécropsie faite 20 semaines après la première injection, furent examinées par microscopie photonique et par immunofluorescence pour la déposition d'immunoglobulines et de complément. Aucune différence significative entre les groupes traités et le groupe témoin ne fut notée dans le ratio protéine:créatinine. Des changements furent observés dans les glomérules par microscopie photonique, mais aucune différence significative entre les différents groupes ne fut mise en évidence par le test exact de Fisher. Le dépôt d'immunoglobulines, mais pas de complément, était significativement plus fréquente* ($P < 0,05$) *dans les glomérules des animaux ayant reçu des injections multiples de vaccin tétravalent que dans ceux ce ayant reçu le vaccin monovalent contre le distemper ou de la saline. Ces résultats suggèrent qu'une vaccination répétée peut augmenter le dépôt glomérulaire d'immunoglobulines. Des études additionnelles sont requises afin de déterminer si l'augmentation de dépôt d'immunoglobulines contribue au développement de dommages glomérulaires et d'identifier les antigènes menant à la production des immunoglobulines déposées.*

(Traduit par Dr Serge Messier)

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Received November 22, 2001. Accepted March 12, 2002.

Introduction

Non-infectious glomerular disease can be classified into 2 major categories: amyloidosis and glomerulonephritis (GN). It is generally accepted that GN is immunologically driven and associated with autoimmune or immune-complex (IC)-mediated tissue injury (1). Immune-complex-mediated GN (ICGN) may result from trapping of antigens in the glomerulus, where interaction of circulating antibody with trapped antigen results in localized IC formation. More commonly, IC-mediated glomerular damage occurs following trapping of preformed circulating immune complexes in the glomerular basement membrane or the mesangium. With continuous or repeated exposure to the inciting antigens, progressive ICGN can develop. Causative antigens can be endogenous or exogenous but are often difficult to identify once bound to antibody and trapped in the glomerulus.

Current knowledge of the pathogenesis of ICGN is derived largely from animal models of the disease. Serum sickness in rabbits, produced by repeated intravenous exposure to foreign antigen, was one of the first models of GN (2). Similarly, ICGN is commonly seen in association with viral infections such as Aleutian disease, in which antibody is non-neutralizing and allows for the persistence of viral antigen (3,4). These models suggest that any situation in which there is repeated antigenic exposure and resultant formation of circulating immune complexes could predispose to the development of GN.

Despite the existence of known infectious causes of glomerular injury in dogs, most cases are classified as idiopathic because the cause is not identified at the time of clinical or pathological diagnosis. Modified live-virus vaccines have been suggested as possible etiologic agents for human ICGN, mostly in case reports (5–9). Repeated vaccination of animals is common and, in the presence of high titers of pre-existing antibodies to the vaccine antigens, could theoretically predispose animals to transient or sustained ICGN. However, there are no published studies of the possible association between this practice and ICGN in animals. Here we describe a study conducted in a mink model to look at the influence of repeated administration of vaccines (particularly modified live-virus vaccines) on the development of ICGN.

Materials and methods

Experimental animals and procedures

Forty-five 10-wk-old male mink were selected from individual litters of a local mink farm, assigned to 3 groups, and housed in individual cages in 1 barn on the farm. Litter selection, individual animal selection, group assignment, and cage allocations were at random, and no litter was represented by more than 1 animal. Serologic testing of randomly selected mink for Aleutian disease and postmortem examination of all mink that died over the preceding 1.5 y indicated that the farm was free of Aleutian disease.

On week 0, all the mink enrolled in the study were sedated by intramuscular (IM) injection of ketamine hydrochloride (Ketaset; Austin Laboratories, Joliette, Quebec) and xylazine-2 hydrochloride (Rompun; Bayvet Division, Chemagro–Haver, Etobicoke, Ontario) to obtain blood samples by cardiac puncture and urine samples by free-flow collection. They were also given a single subcutaneous (SC) dose of a combination vaccine containing aluminum hydroxide gel as adjuvant and modified live canine distemper virus (CDV), inactivated feline parvovirus, botulinum toxoid and a *Pseudomonas* bacterin (Biocom DP; United Vaccines, Madison, Wisconsin, USA).

Subsequently, 15 animals were given 1 mL of sterile saline SC every 2 wk for 16 wk (control group). Another 15 animals received 1 mL of modified live CDV vaccine (Distamink; United Vaccines) SC every 2 wk for 16 wk (distemper group). The 3rd group of 15 animals received 1 mL of the original combination vaccine SC every 2 wk for 16 wk (combination group). Collection of free-flow urine samples was attempted every 2 wk at the same time as vaccination.

During week 20, 4 wk after the last treatment, the mink were sedated as before for blood and urine collection, then euthanized by intracardiac injection of sodium pentobarbital. A complete postmortem examination was performed on all the mink. Kidney samples were fixed in 10% buffered formalin and also embedded in O.C.T. Compound (Sakura Finetek USA, Torrance, California, USA), quick frozen in 2-methylbutane in liquid nitrogen, and stored at −70°C.

All animal procedures were conducted according to the Canadian Council on Animal Care guidelines (10).

Serologic testing

Serum obtained from the blood samples was tested by countercurrent immunoelectrophoresis for antibodies to Aleutian disease virus (11). The remaining portions of the serum were frozen at −20°C and submitted to the Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA, to determine titers of CDV neutralizing antibody.

Urinalysis

The urine samples were examined by routine dipstick urinalysis; if the quantity was sufficient, the sediment was examined microscopically. Samples containing blood due to frequent lower urinary tract infection or crystalluria due to urolithiasis were excluded from the data set (12). Urinary protein and creatinine levels were determined and used to calculate the protein:creatinine (P:C) ratio (12–14).

Histologic and immunopathologic examination of kidney tissues

Histologic sections (5 μm) of kidney were stained with hematoxylin and eosin (HE), Masson trichrome (MTC), and periodic acid–Schiff (PAS) and examined by light microscopy. They were graded as Yes or No for the presence of glomerular lesions, including thickening of the glomerular basement membrane and deposition of mucopolysaccharide and collagen, as evident from the amount and intensity of staining within the glomerular tuft. The sections were examined on 2 occasions, without the examiner knowing the animal identity or the grade previously assigned.

Frozen sections of kidney were examined for the deposition of immunoglobulin G (IgG) and complement by immunofluorescent microscopy (15), following the protocols of the Clinical Immunology Laboratory, Ontario Veterinary College, University of Guelph, Guelph, Ontario. Consecutive sections were stained with

Table I. Titers of neutralizing antibody to canine distemper virus (CDV) in mink following single or repeated vaccination; means are reported on data transformed to negative $\log_2$ values

Group	Treatment[a]	CDV antibody titer (range) Week 0	CDV antibody titer (range) Week 20	Mean titer increase (± SD), weeks 0 to 20	P[b]
Control	Combination vaccine, week 0	< 1/2 to 1/4	< 1/2 to 1/512	5.9 ± 3.48	0.000488
Distemper	Plus 8 doses of CDV vaccine	< 1/2 to 1/8	1/24 to 1/512	7.9 ± 2.15	0.000031
Combination	Plus 8 doses of combination vaccine	< 1/2 to 1/16	1/16 to 1/512	7.6 ± 1.88	0.000244

SD — standard deviation

[a] The combination vaccine contained modified live CDV, inactivated feline parvovirus, botulinum toxoid and a *Pseudomonas* bacterin. The CDV vaccine contained only modified live CDV. After the initial vaccination, mink in the control group were inoculated every 2 wk with sterile saline

[b] Exact *P* values for titer changes from week 0 to week 20, determined by Wilcoxon's signed-rank test. Estimated *P* values for the differences between the means for the treatment and control groups and among the means for the treatment groups are in the Results section

affinity-purified rabbit anti-dog IgG labeled with fluorescein isothiocyanate (FITC) (Jackson Immunochemicals, Westgrove, Pennsylvania, USA) or FITC-labeled rabbit anti-human C3c component of complement (DAKO Corporation, Carpinteria, California, USA). The specificity of the FITC-labeled rabbit anti-dog IgG was assessed by Ouchterlony immunodiffusion and by immunoelectrophoresis. Ouchterlony immunodiffusion showed a line of partial identity between the rabbit anti-dog IgG and mink serum, a line of complete identity with the dog serum, and no cross-reactions with rat serum as a negative control. Following immunoelectrophoresis, prominent identical IgG bands were identified when the rabbit anti-dog IgG was reacted with mink and dog serum. The rabbit anti-human C3c was known to cross-react with mink C3c in rocket immunoelectrophoresis and double immunodiffusion (DAKO, personal communication). In addition, these reagents and the immunofluorescence procedures were assessed with each lot of samples by inclusion of positive- and negative-control frozen sections, prepared, respectively, from the kidneys of mink with and without classic Aleutian disease. Test and control sections were graded as Yes or No for the presence of linear and, or, granular patterns of fluorescence along the basement membrane of multiple glomeruli. As in the histologic grading, the sections were examined on 2 occasions, without the examiner knowing the animal identity or the grade previously assigned.

Statistical methods

For statistical analysis, CDV antibody titers were transformed to negative $\log_2$ values. Because the data were not normally distributed, and the variances were unequal, these data were analyzed by non-parametric tests. To determine whether there was a change in antibody titer from week 0 to week 20, differences between group $\log_2$ CDV antibody titers were assessed by Wilcoxon's signed-rank test, with exact *P* values resulting (16). Additionally, to determine whether the differences in mean titers between the treated groups and the control group and among the treated groups were significant, randomization tests using 1 million random permutations were used (16). Contrasts allow for planned comparison of means between pairs and groups by the use of coefficients of linear comparisons and F-tests as tests of significance (17). Confidence intervals (CIs) for the estimated *P* values were also calculated. Sample-size adequacy was determined by using power calculations for a type I error (α) of 0.05, power of 0.80, when an effect size of 3 on the $\log_2$ transformation scale was considered biologically important (18).

The urinary P:C ratios were transformed to their $\log_{10}$ values to normalize the data. The average P:C ratio for each animal in each group, over all available samplings after the initial vaccination, was used to determine the group median ratios, which were compared by analysis of variance (ANOVA). The assumptions of the ANOVA were adequately met. For sample-size and power calculations, a difference of 1 unit on the original scale was determined to be biologically important. Account was taken of the fact that the data were $\log_{10}$ transformed.

The levels of intra-observer agreement for the histologic and immunopathologic gradings were determined by the κ statistic (19). Individual scores differing on the 2 occasions (i.e., a Yes on 1 occasion and a No on the other) were considered inconclusive and were not included in the subsequent analyses. Overall differences between the proportions of mink in each group with Yes scores for histologic or immunologic lesions were analyzed by Fisher's exact test. When there was a significant difference, pairwise contrasts of proportions of positive gradings were performed, resulting in exact *P* values. Power calculations were based on a difference in proportions when a change from 75% to 25% was considered biologically important (20). The required n for each treatment group was determined (20).

Results

Forty mink remained in the study at week 20. Three animals had died during the study, 2 from cardiac puncture and 1 from undetermined causes. Additionally, 2 mink with positive serologic titers to Aleutian disease were removed from the study. None of the mink showed any clinical abnormalities during the study.

Serologic testing

The titers of CDV neutralizing antibodies increased substantially in most of the mink in each group between weeks 0 and 20. The mean increases in each group were found to be highly significant by Wilcoxon's signed-rank test, the exact *P* values ranging from 0.000031 to 0.000488 (Table I). At week 20, the 2 repeatedly vaccinated groups were found to have higher mean titers than the

Table II. Numbers of mink with glomerular lesions evident in kidney sections stained with Masson trichrome (MTC) and periodic acid–Schiff following vaccination

	Control group		Distemper group		Combination group	
Grade	MTC	PAS	MTC	PAS	MTC	PAS
Yes	6	10	12	11	7	12
No	3	2	1	2	3	0
Inconclusive	4	1	2	2	2	0

Table III. Numbers of mink with glomerular deposition of immunoglobulin G (IgG) and the C3c component of complement evident by immunofluorescent staining of frozen kidney sections following vaccination

	Control group		Distemper group		Combination group	
Grade	IgG	C3c	IgG	C3c	IgG	C3c
Yes	3	0	3	0	9	0
No	8	11	10	14	3	11
Inconclusive	2	2	2	1	0	1

control group by the randomization test ($P = 0.039$, 98% CI = 0.0385 to 0.0394), which indicated that multiple vaccination by either protocol induced higher titers than a single vaccination with the combination vaccine. However, the mean titers of the 2 groups given multiple vaccinations were not significantly different ($P = 0.81$, 98% CI = 0.805 to 0.807). An important difference of 3 on the $\log_2$ transformation scale would be detected with a probability of 80% or better with the sample sizes used.

None of the 45 animals entered in the study had an elevated titer of antibodies to Aleutian disease virus prior to the first vaccination, although 2 animals in the combination group were seropositive for Aleutian disease at week 20. Although these animals did not show typical gross or histopathologic lesions of the disease, their data were removed from the study. False-positive results were suspected.

Urinalysis

Collection of acceptable free-flow urine samples in adequate volumes was difficult owing to the fractious nature of this species and the high frequency of hematuria due to lower urinary tract infection and crystalluria in ranch mink. Consequently, there were insufficient samples to allow analysis of the P:C ratio over each of the samplings. However, inspection of the available individual data and group means did not reveal any consistent changes in the ratio over time, either within or between groups. At the initial sampling, the P:C ratios of the mink in the 3 groups were similar, ranging from 0.41 to 0.66, with means of 0.56 ± 0.14 (standard deviation) for the control group, 0.50 ± 0.03 for the distemper group and 0.59 ± 0.11 for the combination group. At week 20, the P:C ratios ranged from 0.24 to 5.12, with means of 1.16 ± 1.79 for the control group, 0.96 ± 1.0 for the distemper group and 0.74 ± 0.42 for the combination group. The median P:C ratio for each group over all samplings (determined from the average P:C ratios of the individual mink) was 0.67 for the control group, 0.60 for the distemper group and 0.67 for the combination group. One-way ANOVA of these data (following $\log_{10}$ transformation) revealed that there was no treatment effect ($P = 0.89$). Power was about 92%; thus, the sample sizes were adequate to determine the specified differences, as a sample size of 8 per treatment would have given 83.7% power.

Pathology findings

At necropsy, none of the 40 mink showed any gross changes indicative of glomerular disease. Histologic lesions of chronic pyelonephritis were noted in 3 mink in the distemper group.

As there were no consistently identifiable histologic changes in the HE-stained sections of the kidneys, histologic grading was attempted only on sections stained with MTC and PAS. Evident with MTC staining were frequent areas of increased basophilic mesangial staining consistent with collagen deposition. Similarly, diffuse thickening of the glomerular basement membrane was detected with PAS staining. The grades assigned for these sections are given in Table II, which for completeness includes scores that were inconclusive and not included in the statistical analysis. Intra-observer agreement for grading in the 2 blinded examinations of the stained sections was weak to moderate ($\kappa = 55\%$ for the MTC-stained sections and 62% for the PAS-stained sections). Fisher's exact test revealed no significant differences between the proportions of mink with glomerular lesions in each treatment group, as detected by MTC ($P = 0.31$) and PAS staining ($P = 0.52$). With treatment-group sample sizes ranging from 12 to 15, the power of the statistical tests ranged from 60.8% to 68.5% for detecting a true difference of 75% vs 25% in response rates. A sample size of 18 per treatment group would have provided sufficient power (80%).

When present, IgG deposition in the glomeruli was evident mostly as relatively diffuse, linear, yellow/green fluorescence of the basement membrane, present in much of the tuft of all examined glomeruli. There were also focal areas of granularity along the basement membrane in some glomeruli. Agreement between the results of the 2 blinded gradings was moderate ($\kappa = 70\%$). As shown in Table III, glomerular deposition of IgG was most frequent in the combination group (in 9 of the 12 mink). By Fisher's exact test, the proportion of mink with positive immunofluorescence

for IgG was significantly greater in this group than in the control group ($P = 0.022$) or the distemper group ($P = 0.011$). The proportions of mink with positive IgG tests in the control group (3 of 11) and the distemper group (3 of 13) were not significant ($P = 0.84$). With treatment-group sample sizes ranging from 12 to 15, the power of the statistical tests ranged from 60.8% to 68.5% for detecting a true difference of 75% vs 25% in response rates. A sample size of 18 per treatment group would have provided sufficient power (80%). Complement deposition was not consistently apparent in any of the glomeruli of the tested animals (Table III). Also, the intra-observer agreement in the 2 blinded gradings was low ($\kappa = 24\%$). In light of these results, statistical analysis was not attempted.

Discussion

Repeated vaccination of mink with a modified live CDV vaccine or a multivalent vaccine did not cause ICGN that could be detected by the various methods used in this study. There are several possible explanations for this observation, involving related factors that contribute to the development of ICGN. For ICGN to occur, immune complexes must either form in the circulation and become trapped in filtering organs or form in situ in the glomeruli as a result of antigen trapping. "Bystander" injury due to complement fixation and inflammation occurs, resulting in glomerular injury and proteinuria (21,22). The absolute and relative concentrations of antibody and antigen in the circulation are important in the formation of pathogenic soluble circulating immune complexes. Unfortunately, only absolute concentrations of antibody to CDV, which was most readily extrapolated to dogs, were determined in this study. Arguably, it would have been of interest to determine absolute concentrations of antibody to the other components of the 4-way vaccine, particularly the inactivated virus. Additionally, CDV was the only component of the 4-way vaccine that was also available as a separate vaccine product for mink for comparison purposes. Antigenemia, or expression of antigen in renal endothelial or epithelial cells, is similarly important in the development of in situ immune complexes but was not measured in these animals because of the associated technical difficulty.

Repeated intravenous injection of bovine serum albumin has been used to induce glomerular disease in experimental protocols (23,24). Our use of repeated SC injection of modified live-virus vaccine influenced both the degree of hyperimmunization achieved and the levels of antigen reaching the circulation. Titers of CDV antibody in each group increased substantially following vaccination and were significantly higher in the multiply vaccinated groups than in the control group. Although this showed that multiple vaccination produces an increase in antibody titer, it did not confirm that the antibody level achieved was sufficient to result in circulating immune complexes or high antigen levels in the blood. Also, repeated injection of antigen does not necessarily lead to greater immune response, as the total level of antibody in serum is relatively well regulated: even after multiple doses of antigen or exposure to numerous different antigens, the antibody level tends to plateau (15).

Moreover, in this model, after the initial vaccination, the ability of the CDV vaccine strain to replicate and reach the circulation in high concentrations thereafter would almost certainly have been limited by the strong initial antibody response (anamnestic response). Consequently, even if the level of hyperimmunization had been adequate, the level of antigenemia might not have been sufficient to result in the formation of pathogenic immune complexes. The CDV antibody response did not differ significantly between the multiply vaccinated groups receiving CDV singly or in combination; thus, the immunostimulatory effects of the adjuvant did not produce identifiable increases in humoral immune response. Potential differences in cell-mediated responses were not evaluated. Evaluation of the antibody response to the inactivated parvovirus may be prudent.

It is possible that the immunization protocol resulted in circulating immune complexes directed towards a vaccine antigen but that these were not of sufficient magnitude to exceed the kidneys' natural clearance mechanisms. Immunoglobulin or immune complexes may be deposited and subsequently removed by glomerular mesangial cells without producing a complement-driven inflammatory response and subsequent proteinuria (25,26). The 20-wk study duration may not have provided enough time for the development of advanced, easily detectable glomerular lesions, but this period was the complete life span of the commercially owned mink that were available to us. In the case reports involving glomerular disease in humans following multiple vaccination, proteinuria was transient and often detected only within hours to days after inoculation (5,8,23). Similarly, transient IC glomerular injury may have occurred in the mink in this study but been undetected because of the 2- or 4-wk interval between repeat vaccination and urine collection and because of the high rejection rate of urine samples owing to frequent hematuria and, or, hemoglobinuria. The limited numbers of acceptable urine samples precluded analysis of correlates between individual mink over the treatment days. Thus, summary statistics (average ratios) were determined for each animal. The lack of significant differences in the average median urinary P:C ratio suggested that there was no treatment effect.

Two mink were removed from the study because of positive antibody titers for Aleutian disease, a naturally occurring immune-mediated GN caused by parvovirus. However, the farm was negative for the disease, and the seropositive animals did not show typical gross or histologic lesions. Additionally, it has been determined that false-positive results of tests for Aleutian disease can occur in mink tested during the 3-wk period following vaccination against a related parvovirus that causes mink viral enteritis (27). Thus, false-positive results would not be unexpected in mink following the vaccination protocol used in our study.

In the absence of significant, prolonged proteinuria, it was not surprising that we were unable to detect significant glomerular lesions by light microscopic examination of HE-stained slides, as this method is not sufficiently sensitive to detect subtle glomerular lesions of early ICGN. It was hoped that special stains (MTC and PAS) would accentuate subtle lesions. Although microscopic changes were consistently noted in glomeruli from more than 50% of the animals, this was not specific to their vaccination status and, thus, did not appear to be caused by our vaccination protocol. It is possible that the changes detected were within the range of normal variation for juvenile mink. Additionally, it is difficult, in the absence of unvaccinated controls, to determine the effect of the

initial vaccination with the combination vaccine. However, it seems unlikely that a single vaccination could produce histologic changes as severe as those seen following multiple vaccination.

Immunofluorescent microscopy is considered much more sensitive for determining the presence of immune-mediated glomerular injury. In this study, glomerular deposition of immunoglobulin was significantly more frequent in the mink repeatedly vaccinated with the 4-way vaccine than in the mink vaccinated repeatedly with only the distemper vaccine. This suggests that the animals that received the 4-way product had increased formation of in situ immune complexes following glomerular antigen trapping, increased deposition of circulating immune complexes, or greater non-pathogenic accumulation of IgG in the glomeruli. The fluorescence was primarily linear, suggesting in situ IC formation, but this was not confirmed. In addition, the absence of complement deposition or activation suggests that the observed immunoglobulin deposition was not pathogenic or represented transient deposition of immune complexes trapped by normal clearing mechanisms of the mesangial cells. The increased deposition did not appear to be specific for the CDV vaccine, as the most significant deposits occurred in the glomeruli of animals that had received the additional antigens and the adjuvant of the 4-way vaccine. The additional antigens (toxoid, bacterin, and killed virus components) did not require replication to generate their antigenic mass, and 1 or more of these in addition to the adjuvant could have produced a strong IgG response, possibly resulting in the increased glomerular deposition noted. Additional work to determine the target antigen within the kidney would have been useful but was beyond the scope of this preliminary investigation.

Our findings do not strongly support the contention that the repeated use of commercial vaccines is associated with the development of glomerular disease, and this was especially true for the modified live-virus component assessed. Nevertheless, increased deposition of immunoglobulin in the glomeruli of animals that received multiple injections of the 4-way product suggests that this relationship deserves further study. Vaccines containing toxoids, killed bacterial products, and inactivated viral components contain larger antigenic loads and are formulated with adjuvants, which are strong immune stimulatory agents, and so these vaccine products may be more likely to result in hyperimmunization and adverse immune-mediated conditions than modified live-virus products.

There has been much speculation on the role of adjuvants as causative agents in vaccine reactions, the most severe of which are postvaccinal sarcomas. These have been identified in cats following SC or IM injection of both rabies and feline leukemia vaccines (28,29). Current recommendations for cats are to vaccinate every 3 y rather than annually (29). Although protection may require cell-mediated and humoral immunity, antibody titers have been shown to correlate with the level of protective immunity in dogs. As a result, many laboratories are now offering titer determinations to assist in deciding whether vaccination is necessary in a particular year.

Acknowledgments

We acknowledge the kind cooperation of Mr. Thilo Elste and the late Margarete Elste, owners of Cedarbrook Fur Farm, where all the animal studies were performed. Additionally, Dr. Jan Thorsen, Dr. Rob Jacobs, and Dr. Bruce Hunter, from the Ontario Veterinary College, made significant professional contributions. Dr. Mohamed Shoukri assisted with the statistical analysis.

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